

한국인 다낭성 난포증후군 환자에서 5,10-Methylenetetrahydrofolate Reductase의 677번 유전자 다형성에 관한 연구

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The Study of 5,10-Methylenetetrahydrofolate Reductase Variation (MTHFR C677T) in Infertile Females with Polycystic Ovarian Syndrome (PCOS) in Korea

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Objective: To investigate the association of genetic background between MTHFR C677T genotype and infertile females with polycystic ovarian syndrome.

Materials and Methods: We compared 86 infertile females with polycystic ovarian syndrome (PCOS) with 100 healthy fertile females with one or more offspring. Pyrosequencing analysis for MTHFR C677T variation was performed on polymerase chain reaction (PCR) product of study group. To validate pyrosequencing data of C677T variation for randomly selected 50 samples, we compared the pyrosequencing result with the PCR-RFLP (Restriction Fragment Length Polymorphism) result of MTHFR C677T genotype.

Results: The prevalence of the C677T mutant homozygous (TT) was significantly lower ($p=0.0085$) in females with PCOS (8.14%) than in fertile females (21.00%). MTHFR 677 TT genotype had a decreased risk (3.7-fold) of PCOS compared with wild type (MTHFR 677 CC).

Conclusion: Our data support a role for MTHFR mutant homozygous (677 TT) genotype in reducing risk in Korean infertile females with Polycystic ovarian syndrome.

Key Words: Polycystic ovarian syndrome (PCOS), MTHFR, Pyrosequencing

(Polycystic ovarian syndrome, (hyper-
PCOS) androgenism), (chronic anovulatory
가 . cycles), 가

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This work was supported by the Samsung grant, #SBRI C-A2-313-1.

¹ 가 , Orio ¹⁶
(LH) MTHFR 677
(FSH) 가 가
^{2,3} 677 가

가
INS VNTR (Insulin gene variable number of tandem repeats)⁴ CYP11 α ⁵
(folate metabolism) 86
MTHFR 1 가
. MTHFR 100 .
(colorectal cancer)⁶, (Cardiovascular disease)⁷ 가
MTHFR 677 (hyperandrogenism), , , 가 LH, LH/FSH 가 2~3 , (sonographic finding) ,

, MTHFR C677T (preeclampsia), (intrauterine growth retardation; IUGR), (stillbirth)
⁸ , MTHFR ¹⁷
677 , ⁹ ,
MTHFR 2.
(recurrent pregnancy loss) 가 MTHFR 677
^{10,11} (folate metabolism) (enzyme)
MTHFR (methylenetetrahydrofolate reductase) 5, 10-methylenetetrahydrofolate 5-methyltetrahydrofolate (5-methyl-THF)
, MS (methionine synthetase) (PCR) 1298
(methionine) (methyl) 113 bp가
(annealing temperature) 55
45 . 2% agarose
MTHFR C677T gel ethidium bromide
가 , 677 (biotin)
(TT) pyrosequencing
가 ¹²⁻¹⁴ 가 (sequence primer) 5'-GGT GTC TGC GGG A-
DNA, (protein), (lipid) 3' ¹⁸
SAM (S-adenosylmethionine) 가 DNA hypomethylation Pyrosequencing 50 DNA ,
, DNA ¹⁵
¹⁵ (Restriction Fragment Length Polymorphism)

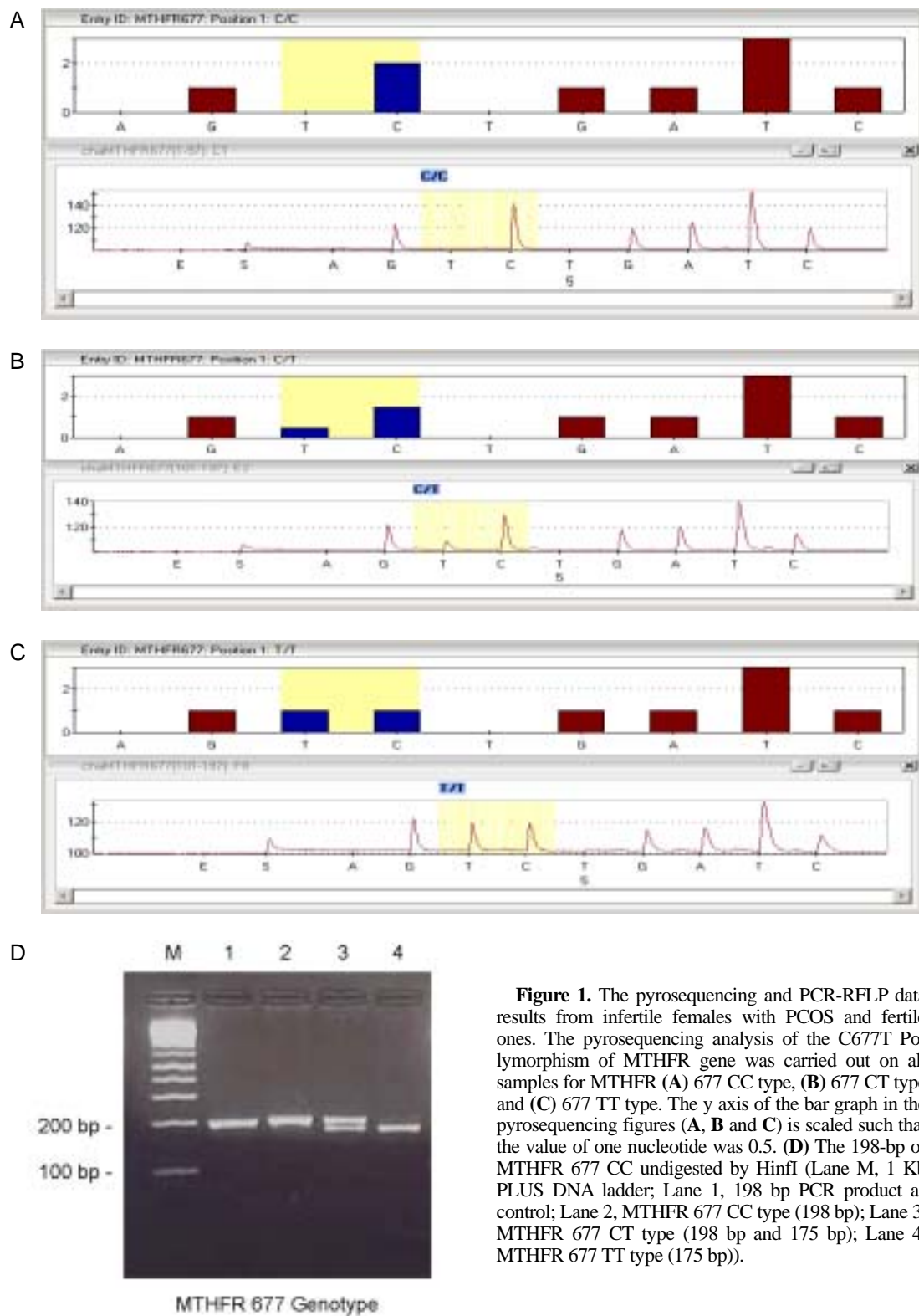


Table 1. Distribution of the genotypes of MTHFR (5,10-methylenetetrahydrofolatereductase) 677 in fertile female and PCOS (polycystic ovary syndrome) patients

	Female control (n=100)	PCO patient (n=86)	OR (95% CI)	p
677 CC	27.00% (n=27)	38.37% (n=33)	-	-
677 CT	52.00% (n=52)	53.49% (n=46)	-	-
677 TT	21.00% (n=21)	8.14% (n=7)	0.2727 (0.10~0.74)	0.0085
CT + TT	73.00% (n=73)	61.63% (n=53)	-	-
T allele	47.00% (n=94)	34.88% (n=60)	0.6041 (0.40~0.91)	0.0180

OR=odds ratio; CI=confidence interval

677 forward primer: 5'-TGA AGG AGA AGG TGT CTG CG-3' reverse primer: 5'-AGG ACG GTG CGG TGA GAG TG-3' PCR machine (MJ research thermal cycler, Waltham, USA) 198 bp 95 primer (annealing) 61 30 primer extension 72 30 primer 35 677 A C HinfI (New England Biolabs, Beverly, MA, USA) 37 Alanine valine 175 bp 23 bp 3% 7† ethidium bro- mide 3. SAS (SAS Insti- tute, Cary, NC) (SAS Insti- tute, Cary, NC) (Chi-square) (Fisher's exact) odd ratio (OR) 95% (95 percent confi- dence intervals, 95% CI) 100 (pyro- sequencing 5,10-methyleneTHF

MTHFR 677 7† cyto- sine thymine Pyrosequencing RFLP Figure 1 Table 1 MTHFR 677 CC, CT, TT type 38.37%, 53.49%, 8.14%, 27.00%, 52.00%, 21.00% 677 TT type (p=0.0085)7† 677 TT type 7† 3.7 (OR=0.2727, 95% CI=0.10~0.74; p=0.0085) , T (allele) (OR=0.6041, 95% CI=0.40~0.91; p= 0.0180).

677 TT 3.7 (Table 1; p=0.0085). , 677 TT 677 TT

misincorporation" MTHFR 가 DNA "uracil

, MTHFR 677 dUMP가 dTMP 5,10-methyleneTHF dTMP DNA (acute lymphocytic leukemia)²⁰ (proximal colon cancer)²¹ 가 MTHFR 가 , 677 TT 가 , .¹⁶ MTHFR 677 DNA 가 MTHFR 가 ,²²

1. Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995; 333: 853-61.
2. Adams J, Franks S, Polson DW, Mason HD, Abdulwahid N, Tucker M, et al. Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. *Lancet* 1985; 2: 1375-9.

3. Conway GS, Honour JW, Jacobs HS. Heterogeneity of the polycystic ovary syndrome: clinical, endocrine and ultrasound features in 556 patients. *Clin Endocrinol (Oxf)* 1989; 30: 459-70.
4. Waterworth DM, Bennett ST, Gharani N, McCarthy MI, Hague S, Batty S, et al. Linkage and association of insulin gene VNTR regulatory polymorphism with polycystic ovary syndrome. *Lancet* 1997; 349: 986-90.
5. Diamanti-Kandarakis E, Bartzis MI, Bergiele AT, Tsianateli TC, Kouli CR. Microsatellite polymorphism (tttta) (n) at -528 base pairs of gene CYP11 alpha influences hyperandrogenemia in patients with polycystic ovary syndrome. *Fertil Steril* 2000; 73: 735-41.
6. Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* 1996; 56: 4862-4.
7. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. *Am J Clin Nutr* 2000; 72: 324-32.
8. Kupfermanc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med* 1999; 340: 9-13.
9. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *Am J Clin Nutr* 1999; 70: 495-501.
10. Candito M, Magnaldo S, Bayle J, Dor JF, Gillet Y, Bongain A, et al. Clinical B12 deficiency in one case of recurrent spontaneous pregnancy loss. *Clin Chem Lab Med* 2003; 41: 1026-7.
11. Pauer HU, Voigt-Tschirschwitz T, Hinney B, Burfeind P, Wolf C, Emons G, et al. Analyses of three common thrombophilic gene mutations in German women with recurrent abortions. *Acta Obstet Gynecol Scand* 2003; 82: 942-7.
12. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard

- CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; 10: 111-3.
13. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996; 93: 7-9.
 14. Kluijtmans LA, Kastelein JJ, Lindemans J, Boers GH, Heil SG, Bruschke AV, et al. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. *Circulation* 1997; 96: 2573-7.
 15. Blount BC, Ames BN. DNA damage in folate deficiency. *Baillieres Clin Haematol* 1995; 8: 461-78.
 16. Orio F Jr, Palomba S, Di Biase S, Colao A, Turchmanova L, Savastano S, et al. Homocysteine levels and C677T polymorphism of methylenetetrahydrofolate reductase in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2003; 88: 673-9.
 17. Kim NK, Nam YS, Ko JJ, Chung HM, Chung KW, Cha KY. The luteinizing hormone beta-subunit exon 3 (Gly102Ser) gene mutation is rare in Korean women with endometriosis and polycystic ovary syndrome. *Fertil Steril* 2001; 75: 1238-9.
 18. Ahmadian A, Gharizadeh B, Gustafsson AC, Sterky F, Nyren P, Uhlen M, et al. Single-nucleotide polymorphism analysis by pyrosequencing. *Anal Biochem* 2000; 280: 103-10.
 19. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* 1998; 64: 169-72.
 20. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc Natl Acad Sci USA* 1999; 96: 12810-5.
 21. Toffoli G, Gafa R, Russo A, Lanza G, Dolcetti R, Sartor F, et al. Methylenetetrahydrofolate reductase 677 C --> T polymorphism and risk of proximal colon cancer in north Italy. *Clin Cancer Res* 2003; 9: 743-8.
 22. Brattstrom L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. *Circulation* 1998; 98: 2520-6.
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